REMARKS

I. The claims

Pursuant to this paper, claims 1-38 are pending. Claims 1-16 are presently withdrawn as a result of being deemed to be directed to a non-elected inventions and claims 21, 23, 26, 28, 31 and 33-35 are presently withdrawn as being drawn to non-elected species.

Elected claims 17-20, 22, 24, 25, 27, 29, 30, 32 and 36 were examined on the merits and were rejected in the Final Office Action mailed August 29, 2007, but were previously found to be free of the prior art. (First Office Action, ¶7.)

New dependent claims 37 and 38 have been added herein, read on the presently elected invention, and specify that the cells recited in respective base claims 17 and 18 are transgenic plant cells. Support for new claims 37 and 38 is found, for example, in the originally filed specification at page 3, l. 28-29 and in originally filed claim 36.

No new matter has been added by any of the amendments made herein.

II. Claim rejections under 35 U.S.C. §112 – enablement requirement

The Examiner maintained the rejections of the pending elected claims for alleged lack of enablement under 35 U.S.C. §112, paragraph 1. (Final Office Action, ¶4.) The Examiner's assertions regarding the alleged nonenablement fall into two broad categories: (A.) alleged nonenablement of gene targeting/random integration aspects (Final Office Action, page 2, l. 15 to page 8, l. 2); and (B.) alleged nonenablement of RNA silencing (RNA interference, RNAi) aspects (Final Office Action, page 8, l. 3 to page 8, line 20).

A. Response to aspects of rejection for alleged non-enablement of gene targeting/random integration

The Examiner's bases of rejection regarding the alleged nonenablement of gene targeting/random integration aspects (Final Office Action, page 2, l. 15 to page 8, l. 2) are

identical to those presented by the Examiner in parent application serial no. 10/354,903 ("the parent application"). As the Examiner is aware, Applicant filed a Pre-Appeal Brief Request for Review on October 31, 2006 in the parent application, including Applicant's Pre-Appeal Brief Arguments. A Notice of Panel Decision from Pre-Appeal Brief Review withdrawing said rejections in the parent application was issued December 14, 2006. Accordingly, Applicant submits that the present bases of rejection for alleged lack of enablement that concern gene targeting/random integration aspects should be withdrawn in view of Applicant's prior Amendment and Response filed June 13, 2006 in this application and the referenced panel decision in the parent application. For the sake of brevity, Applicant has submitted, in the enclosed IDS for consideration by the Examiner, a copy of each of the complete Pre-Appeal Brief Request for Review filed in the parent application (the arguments of which should be considered to be incorporated by reference herein) and the Notice of Panel Decision from Pre-Appeal Brief Review issued in connection therewith.

B. Responses to aspects of rejection for alleged non-enablement of RNA silencing

(B1.) Examiner's assertions based on Sledz et al. (2005)

The Examiner has asserted that Sledz et al. (2005; "Sledz") is a proper basis in showing nonenablment of the presently claimed invention since, even though Sledz is predominantly directed to gene therapy applications of RNAi, "delivery concerns and non-specific effects still apply if the invention is practiced in a living organism" and the current claims encompass "in vitro, ex vivo or in vivo" situations." (Final Office Action, page 8, l. 3-10)

The present basis of rejection is traversed for the following reasons.

The passage of Sledz originally cited for delivery concerns by the Examiner in the nonfinal Office Action mailed February 13, 2006 ("First Office Action"), specifically relates to alleged stability issues in circulation, i.e., in the circulation of *animals*: "[t]he use of RNAi for therapeutic purposes will depend on other factors as well…[a]lthough siRNAs are relatively stable in cell culture conditions, they require enhanced nuclease and thermodynamic stability when in circulation *in vivo*." (First Office Action, page 10, l. 17-19.)

Plants. First, Applicant wishes to point out that the alleged concern, in any case, does not apply to plants. Sledz is clearly directed to RNA silencing of animal cells and the goal of gene therapy in animals/humans and the "*in vivo* circulation" referred to in Sledz unambiguously refers to animal circulatory systems, i.e., primarily blood circulation. The situation in plants is entirely different.

Further, although the claims do not require that the RNA silencing-induced (recombinase-mediated) DNA-excision must spread through a whole plant, Applicant wishes to point out that the intercellular spreading of RNA silencing signals is one of the hallmarks of RNA silencing in plants. See, e.g., Waterhouse et al. (2001), Nature 411: 834-842, at page 838, section entitled "PTGS can spread systemically through a plant;" and ‚Jorgensen et al. (1998) Science 279: 1486-1487, entire document, each of which is of record in the instant application.

In further connection with plants, *efficient* methods for inducing gene silencing are well known. For example, as pointed out previously Amendment and Response filed June 13, 2006, (on page 22), Wesley et al. (2001) Construct design for efficient, effective and high-throughput gene silencing in plants, The Plant J. 27(6), 581-590, which is of record in the application, teaches efficient methods for silencing selected targets in plants. See also, Angell et al. (1997) Consistent gene silencing in transgenic plants expressing a replicating potato X RNA, EMBO, 16(12); 3675-3684 and Gossele et al. (2002) SVISS – a novel transient gene silencing system for gene function discovery and validation in tobacco plants, The Plant J. (32): 859-866.

In connection with the same, MPEP 2164.04 states that "the examiner should always look for enabled, allowable subject matter and communicate to applicant what that subject matter is at the earliest point possible in the prosecution of the application." In this regard, in addition to Applicant's request for complete withdrawal of the present rejections for alleged lack of enablement in view of all the reasons presented herein, Applicant also specifically requests that the Examiner provides an indication that the presently claimed invention is enabled for plants and other specific systems. Originally filed claim 36 recites a transgenic plant and each of new dependent claims 37 and 38 recite transgenic plant cells.

Animals. Notwithstanding the above, the cited passages of Sledz on which the Examiner has relied do not support the alleged nonenablement of the presently claimed invention in animals for the following reasons.

First, *in vivo* RNA silencing in animals was readily obtainable as shown for mice and *Drosophila* two preeminent model organisms –in the following references:

1. Lewis et al. (2002), Efficient delivery of siRNA for inhibition of gene expression in postnatal mice, Nat. Genet. 32: 1107-1108 (newly submitted in IDS);
2. Song et al. (2003) RNA interference targeting Fas protects mice from fulminant hepatitis, Nat. Med. 9: 347-351 (newly submitted in IDS);
3. McCaffrey et al. (2002) RNA interference in adult mice, Nature 418: 38-39 (newly submitted in IDS); and
4. Piccin et al. (2001) Efficient and heritable functional knock-out of an adult phenotype in Drosophila using a GAL4-driven hairpin RNA incorporating a heterologous spacer, Nuc. Acid Res. 29(12) e55 page 1-5 (previously of record).

Indeed, even Woessmann et al. (2003), which the Examiner has also cited for bases of rejection, teaches that "effective siRNA are usually identified at high frequency not only for analyzing cultured cells but also for the induction of RNAi in mice." (Woessmann et al. (2003), page 274, right-column, l. 18-21; citing documents 1 through 3 listed above; emphases added.)

The Examiner has also alleged that "non-specific effects" would still be a problem if the presently claimed invention is practiced in an organism, i.e., *in vivo*. (Final Office Action, page 8, l. 3-6.) Applicant has already pointed out that non-specific effects are not necessarily a problem so long as the desired specific effect is obtained. (Amendment and Response filed June 13, 2006, page 24, l. 20-24.) The Examiner's rationale is not clearly understood, but it appears that the basis of the Examiner's statement is the presupposition that non-specific effects will be *unhealthy* to an animal subject. First of all, whether there would be side-effects caused by non-specific effects in a non-human animal has no bearing on patentability whatsoever. Even in a human being, it is well known that therapeutics such as cytotoxic cancer agents generally have non-specific effects, harming normal cells. Second, the presently claimed invention, of

course, does not cover genetically engineered human beings so non-specific side effects are not an issue here either. Third, the Examiner seems to again be imposing an improper standard of optimization that is above and beyond that required for enablement, i.e., that there must be no non-specific effects. Non-specific effects are something that occurs in addition to specific effects - as long as the desired effect occurs, there is enablement. Indeed, as set forth in MPEP 2164, "to comply with 35 U.S.C. 112, first paragraph, it is not necessary to enable one of ordinary skill in the art to make and use a perfected, commercially viable embodiment absent a claim limitation to that effect." Further in this regard, Applicant wishes to point out that the certain statements in the Examiner's cited passage of Sledz are given in the context of finding optimization and do not present the particular issues as barriers to silencing: " [i]n addition, the half-life of the target message and/or protein needs to be considered in order to achieve optimal silencing." (First Office Action, page 10, l. 15-16.)

Finally, Applicant urges the Examiner to reconsider Applicant's remarks concerning Sledz on pages 22-23 of Applicant's Amendment and Response filed June 13, 2006.

(B2.) Examiner's assertions based on Woessmann et. al. (2003)

The Examiner has continued to assert Woessmann et al (2003; "Woessmann") as a basis for the present rejection and, in response to Applicant's Amendment and response filed June 13, 2006, has now stated that the alleged problem associated with the RNA editing process is not overcome by using a mini-gene since "the RNA editing machinery alters mRNA sequences after transcription from the genomic template." (Final Office Action, page 8, l. 11-17.)

The presently asserted basis of rejection is overcome for the following reasons.

The passages of Woessmann relied upon by the Examiner appear in the First Office Action at page 11, lines 3-13, wherein four specific phenomena are set forth, namely: (1.) "a single point mutation in the targeted fusion site may abolish siRNA-mediated mRNA-degradation of the oncogene," (2.) mutations in proteins of the RNAi machinery, e.g., the argonaute proteins, could also render tumor cells resistance to RNAi," (3.) "an amplification of the fused oncogene, recently also demonstrated in

BCR/ABL-positive leukemias after treatment with the tyrosine kinase inhibitor imatinib, may also result in an inefficient fusion gene suppression," and (4.) "RNAi could also be antagonized by a mechanism called RNA editing". (The full passage of Woessmann entitled "Proposed Mechanism for RNAi Resistance" is found on page 284, right-column, l. 28 to page 285, l. 13 of the reference.)

Before addressing each of the points from the passage that were relied on by the Examiner, Applicant again wishes to point out that Woessmann itself provides abundant evidence for the enablement of RNA silencing of selected genes, *including silencing of oncogenes*. Table 3 on pages 278 and 279 of Woessman cites references describing the silencing of 14 different oncogenes of 11 different types and the delivery methods used. Moreover, as also pointed out above, Woessmann explicitly teaches that "effective siRNA are usually identified at high frequency not only for analyzing cultured cells but also for the induction of RNAi in mice." (Woessmann, page 274, right-column, l. 18-21; citing documents 1-3 listed above; emphases added.)

Woessmann passage, points 1 through 3. The Examiner's present statements in the Final Office Action appear to concede that the first three cited statements of Woessmann are no longer a basis of rejection, i.e., were traversed by Applicant's previous observations and arguments. Nevertheless, with respect to points (1.) through (3.) from the cited passage of Woessmann, Applicant wishes to point out the following. In connection with point (1.), it is noted that that the term "fusion gene" unambiguously refers to fusion-type oncogenes in Woessmann. (See Woessmann, page 281 left-column, l. 1 to page 282, right-column, l. 30.) It is well known that tumors cells exhibit hyper-mutatability. The presently claimed invention involves RNA silencing of a preselected repressor protein that is operably linked to a recombinase gene expression element. The presently claimed situation is entirely different and remote from the situation of silencing an oncogene in tumor cells. In connection with point (2.), while this hypothetical phenomenon might be of some relevance to treating a tumor in a therapeutic context, it is not seen how it can possibly be relevant to the presently claimed invention. In a therapeutic tumor treatment situation, mutation in some cells could potentially give rise to cells that are resistant to an RNAi treatment. The Examiner should be aware that this type of "escape" is a general phenomenon with almost any type of anti-cancer therapy

including conventional chemotherapies. Although a therapy may indeed be effective in almost all tumor cells (and therefore operative), resistant mutant cells in the tumor may escape giving rise to a resistant tumor. The same situation occurs with chemotherapy of HIV. Even if such a mutation were to occur in the context of the presently claimed invention – which is a totally hypothetical event – it would not occur in every cell of an organism and it would not occur in all cells of a cell culture. Thus, asserted point (2.) has no bearing whatsoever on enablement. Point (3.) from the Woessmann passage relates to amplification of an oncogene – again this situation is entirely different and remote from the genetic situation of the presently invention wherein the silenced gene is a preselected repressor operably linked to a recombinase expression element.

Woessman passage, point 4. Applicant has carefully reanalyzed the teachings of Woessmann regarding point (4.) which was relied on by the Examiner, including the underlying references. As taught by Woessmann on page 285 "RNA editing involves adenosine deaminases," which "enzyme creates inosines by deamination of adenosines in dsRNA." The specific concern raised in Woessmann is that "RNA editing could antagonize RNAi through the inhibition of the recognition of siRNA by the RNA machinery." (Id.) Thus, what is referred to *is* the potential editing of the siRNA molecule itself.

First, Applicant again wishes to point out that the "concern:" of point (4.) of the Woessmann passage is given entirely in the context of the potential for resistance to RNAi *developed by a tumor in the context of therapy.* As pointed out above, Table 3 of Woessmann clearly shows that silencing of 14 different oncogenes of 11 different type s was achieved by workers in the field. Thus, the concern is not born out and, to whatever extent it may have merit, it is an issue of tumor cells "escaping" therapy as described above. Such potential for escape does not bear whatsoever on the enablement of the present invention, nor on any number of patented therapeutics for treatments cancers and viral pathogens such as HIV.

Second, notwithstanding the above, the underlying references for point (4.) of the cited Woessmann passage indicate that the alleged concern of "editing" of siRNA molecules applies to "hyper-editing" situations and is not a general phenomenon. For example, underlying reference Scadden et al. (2001) RNAi is antagonized by A→I hyper-

editing, EMBO reports 21(121): 1107-1110 (disclosed in accompanying IDS), teaches at page 1110, right-column, that:

> Expression of ADARs [adenosine deaminases that act on dsRNA] in various organisms is highest in neural tissues and also in the developing vulva of *C. elegans* (L. Tonkin and B. Bass, personal communication). RNAI in most *C. elegans* tissues can be readily achieved by soaking the worms in dsRNA or feeding with *Escherichia coli* strains that express a trigger dsRNA. In contrast, neurons are relatively resistant (Kamath *et al.,* 2000; Maeda *et al.,* 2001), and efficient RNAi of neuronally expressed genes has only been achieved by *in vivo* expression of a heritable inverted repeat gene corresponding to the target gene under the control of a strong heat shock-inducible promoter (Tavernarakis *et al.,* 2000). Likewise, injection of dsRNA was unable to invoke RNAi in the vulva (Fire *et al.,* 1998).

Note that the above cited passage also indicates that the resistance can be overcome (sentence citing Tavernarakis), *specifically providing the guidance* that use of a strong promoter can be used to overcome the resistance.

Further, Scadden et al. (2001), specifically teaches where (in what tissue types) such hyper-editing might be considered to pose a problem. Thus, since the prior art specifically provides guidance when (in what tissue types), the alleged cases of inoperability cannot be considered to support the asserted nonenablement. As set forth in MPEP: 2164.08(b) Inoperative Subject Matter (emphasis added):

> The presence of inoperative embodiments within the scope of a claim does not necessarily render a claim nonenabled. The standard is whether a skilled person could determine which embodiments that were conceived, but not yet made, would be inoperative or operative with expenditure of no more effort than is normally required in the art. Atlas Powder Co. v. E.I. du Pont de Nemours & Co., 750 F.2d 1569, 1577, 224 USPQ 409, 414 (Fed. Cir. 1984) (prophetic examples do not make the disclosure nonenabling).

Accordingly, in connection with the Examiner's RNA editing rationale for rejection, since the prior art provides specific guidance as to the specific types of tissues in which RNA hyper-editing could present a problem for RNAi, a skilled worker would know, *a*

priori, which embodiments would be operable and inoperable. Therefore, the proposed bases for nonenablement presented by the Examiner must be withdrawn.

(B3.) Examiner's assertions based on Opalinska et al. (2002)

The Examiner has maintained the basis of rejection that relies on Opalinska et al. (2002; "Opalinska"). Specifically, the Examiner has now asserted that "it is not clear why the reference is questionable, since considerations that apply to nucleic acids, in particular anti-sense oligonucleotides also apply, since siRNA mediate the silencing of target mRNAs." (Final Office Action, page 8, l. 18-20.)

The asserted basis of rejection is overcome for the following reasons:

First, the Sledz and Woessmann references cited by the Examiner as well as Applicant's cited references, as discussed hereinabove, provide affirmative teachings that specific RNA silencing can usually be obtained at high frequency and provide numerous examples. The weight of the references including those cited by the Examiner contradict the conclusion drawn by the Examiner from Opalinska. Even on this basis alone, reliance on Opalinska should be withdrawn.

Second, the Examiner's rationale for continuing to rely on Opalinska is improper because what *is* widely known in the art is that there are substantive differences between antisense oligonucleotide technology, which relies on single-stranded DNA and single stranded-DNA derivatives while RNA silencing depends (*as in the present claims*) on use and/or formation of double-stranded RNA. The Examiner's conclusion, "consideration that apply to nucleic acids, in particular anti-sense oligonucleotides also apply, since siRNA mediate the silencing of target mRNAs." is not supported by a specific technical reason and ignores the art-recognized differences between conventional anti-sense technology and RNA silencing technology. The Examiner's burden is to provide a specific technical basis supported by evidence, which has not been provided here. See MPEP 2164.04 Burden on the Examiner Under *>the< Enablement Requirement [R-1] (emphases added):

> While the analysis and conclusion of a lack of enablement are based on the factors discussed in MPEP § 2164.01(a) and the evidence as a whole, it is not necessary to discuss each factor in the written enablement rejection. The language should focus on those factors,

> reasons, and evidence that lead the examiner to conclude that the specification fails to teach how to make and use the claimed invention without undue experimentation, or that the scope of any enablement provided to one skilled in the art is not commensurate with the scope of protection sought by the claims. This can be done by making specific findings of fact, supported by the evidence, and then drawing conclusions based on these findings of fact. For example, doubt may arise about enablement because information is missing about one or more essential parts or relationships between parts which one skilled in the art could not develop without undue experimentation. In such a case, the examiner should specifically identify what information is missing and why one skilled in the art could not supply the information without undue experimentation. See MPEP § 2164.06(a). References should be supplied if possible to support a *prima facie* case of lack of enablement, but are not always required. *In re Marzocchi*, 439 F.2d 220, 224, 169 USPQ 367, 370 (CCPA 1971). However, specific technical reasons are always required.

Third, in specific connection with the Examiner's present assertion that "it is not clear why the reference is questionable" Applicant wishes to again point out the remarks presented on pages 22 to 24 of the Amendment and Response filed June 13, 2006 ("First Reply"), where Applicant specifically points out that the cited passages of Opalinska rely on outdated underlying references and/or are directed to conventional antisense technology (First Reply, page 23, l. 6-22) and present a conclusion that is blatantly illogical and cannot be properly drawn (First Reply, page 23, l. 23 to page 24, l. 2). The illogical conclusion and unsupported conclusion cannot be properly relied upon for the present rejection and call into question the authoritativeness of the reference, both specifically and generally. Applicant very specifically described on page 23, l. 6-22 of the First Reply that the references of Opalinska that underlay the statement of Opalinska that the Examiner relies on, namely references no. 120 (Gerwitz et al. 1996, Proc. Nat'l Ac. Sci. USA 93: 3161-3163) and no. 121 (Lebedeva et al. 2001, Annu. Rev. Pharmacol. Toxicol. 41: 403-419) relate to conventional DNA-based antisense technology, have nothing whatsoever to do with RNAi, and are outdated (especially in the case of Gerwitz et al., 1996). Thus, it is plain error for the Examiner to rely on the statement of Opalinska that is supported only by these underlying references. Since the Examiner was apparently not convinced by Applicant's prior arguments in this regard, in further support Applicant has submitted a copy of each of Gerwitz et al. (1996) and Lebedeva et al., (2001) with the

accompanying Information Disclosure Statement for further review by the Examiner. A review of the two articles will demonstrate that they unambiguously relate only to conventional DNA-oligonucleotide antisense technology and conventional modifications thereof, and not to RNAi whatsoever.

Of course, Applicant's observations in the previous paragraph, are *also in addition* to the fact that the Examiner's assertion based on Opalinska contradicts the teachings of Sledz et al. (2005), Woessmann et al. (2003) and the references cited by Applicant that support enablement, as discussed above. Thus, the situation is that the RNAi-related references that the Examiner has cited actually support enablement of the RNAi aspect of the present invention, while the statements of Opalinska that the Examiner has relied on have nothing at all to do with RNAi and, thus, are profoundly less significant than the RNA-related references which support enablement. Moreover, as also previously pointed out in Applicant's First Reply, the Examiner's reliance on the cited passage of Opalinska fails to take into account the ability to express silencing RNAs within cells and other art-recognized differences with conventional antisense technology (First Reply, page 24, 3-9).

As set forth in MPEP 2164.05, "the determination [of enablement] should always be based on the weight of all the evidence." As set forth in MPEP 2164.08, "the scope of enablement must only bear a 'reasonable correlation' to the scope of the claims." Applicant has addressed each and every part of the Examiner's asserted Wand's analysis and conclusions in connection therewith by showing that the cited references were improperly relied upon by the Examiner and/or by showing enablement of the specific aspects of the invention with reference to the documents previously and now of record. In view of Applicant's arguments and the evidence discussed above, the weight of the evidence supports a finding of enablement with respect to both the recombination/gene targeting aspects and the RNA silencing aspects of the invention and withdrawal of the claim rejections under 35 U.S.C. §112, paragraph 1 – enablement requirement is therefore requested

III. Conclusion

Claims 1-38 are pending. Claims 1-16 are presently withdrawn as a result of being deemed to be directed to a non-elected inventions and claims 21, 23, 26, 28, 31 and 33-35 are presently withdrawn as being drawn to non-elected species. .

Pursuant to this paper, Applicant submits that elected claims 17-20, 22, 24, 25, 27, 29, 30, 32 and 36 and new claims 37 and 38 (reading on the elected invention) are in condition for further examination and allowance, which action is hereby requested. Upon a finding of allowance for any of the elected claims, Applicant requests rejoinder and allowance of any presently withdrawn claim that is dependent on an allowed elected claim. If upon considering this paper, the Examiner still considers any claim to be unallowable, Applicant respectfully requests that the Examiner telephone Applicant at the number below to discuss any issues that may remain.

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